

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
24 July 2003 (24.07.2003)

PCT

(10) International Publication Number
WO 03/060072 A2

(51) International Patent Classification⁷: C12N
(21) International Application Number: PCT/US02/40913
(22) International Filing Date:
20 December 2002 (20.12.2002)
(25) Filing Language: English
(26) Publication Language: English
(30) Priority Data:
60/343,315 21 December 2001 (21.12.2001) US
(71) Applicant (for all designated States except US): IMMUNEX CORPORATION [US/US]; Law Department, 51 University Street, Seattle, WA 98101 (US).

(81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

(72) Inventor; and
(75) Inventor/Applicant (for US only): REDDY, Pranhitha [US/US]; 6811 18th Avenue NE, Seattle, WA 98115 (US).
(74) Agents: ODRE, Steven, M. et al.; Immunex Corporation, Law Department, 51 University Street, Seattle, WA 98101 (US).

Published:

— without international search report and to be republished upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 03/060072 A2

(54) Title: RECOMBINANT POLYPEPTIDES

(57) Abstract: The present invention relates, in part, to a polypeptide domain having reduced immunogenicity that is capable of inducing trimers in heterologous polypeptides, fusion polypeptides comprising the trimer inducing domain, nucleic acids encoding the polypeptides of the invention and methods of making and using the same.

RECOMBINANT POLYPEPTIDES

This application claims the benefit of U. S. Provisional Application No. 60/343,315, filed December 21, 2001 which is hereby incorporated by reference.

5

INTRODUCTION

The present invention is directed, in part, to compositions relating to polypeptide domains having reduced immunogenicity that are capable of inducing trimers in heterologous polypeptides, fusion polypeptides comprising such trimer inducing domains, nucleic acids encoding the polypeptides of the invention, and methods of making and using the same.

10

BACKGROUND

The structure of a polypeptide is an important element in its activity. Oftentimes, a properly folded polypeptide needs to associate with itself or different polypeptides in order to be active. Most often these interactions are as homodimers and provide an active complex, however, heterotrimers also occur. Defined trimerizing motifs are typically coiled coil domains (Burkhard et al., 2001, Trends in Cell Biol., 11:82), and includes SP-D trimerizing domains (U.S. Patent Nos. 5,716,805 and 6,190,886).

15

Although lacking a distinctive coiled coil motif, additional molecules known to require trimerization for optimal activity include the tumor necrosis factor super family, such as TNF-alpha, lymphotoxin alpha, and CD40L (TNFSF5), among others. Without trimerization, many TNF super family polypeptides have low or absent activity (Fanslow et al., (1994) Seminars Immunology, 6:267-278). CD40 is a transmembrane polypeptide member of the tumor necrosis factor receptor super family and is involved in stimulating proliferation and differentiation of humoral and cellular immune cells. In particular, CD40 is involved in isotype switching and is important in T-cell activation and production of type 1 cytokines in response to protein antigens (Noelle, R, (1996) Immunity, 4:415; and Borrow et al., (1996) J. Exp. Med., 183:2129).

20

25

It has been shown that CD40L (TNFSF5) is normally active in a membrane bound form, however, this form is difficult to administer as a therapeutic. The soluble version of

30

CD40L (TNFSF5) was found to have very little stimulatory activity, but by trimerizing soluble CD40L (TNFSF5) using a mutated GCN4 leucine zipper domain, significant activation of target cells was achieved (Fanslow et al., (1994) *Seminars Immunology*, 6:267-278; Morris et al., (1999) *J. Biol. Chem.*, 274:418-423; U.S. Patent No. 5,716,805).

A significant limitation of using the mutant leucine zipper trimerizing domain in a therapeutic fusion polypeptide is that leucine zippers are typically nuclear proteins, *i.e.*, intracellular. Thus even though the polypeptide may be of the same species as a subject being treated with the polypeptide, having not been seen by the immune system can lead to the polypeptide being recognized as foreign when expressed extracellularly and triggering an immune response in the subject. This response is particularly deleterious to the continuous or long-term administration of the fusion polypeptide to a patient. The present invention addresses this issue by providing polypeptide domains that will have reduced immunogenicity in addition to having trimerization properties.

15 SUMMARY OF THE INVENTION

The present invention relates, in part, to a polypeptide that is capable of inducing trimerization when fused to a heterologous polypeptide, fusion polypeptides comprising the trimerizing domain, nucleic acids encoding the polypeptides of the invention and methods of making and using the same. More particularly, the present invention is directed to a fusion polypeptide comprising a first polypeptide capable of forming a trimer fused to a second heterologous polypeptide having a desired biological activity. It is also contemplated that the fusion of a first polypeptide capable of forming a trimer and a second polypeptide heterologous to the first polypeptide results in an increase in the biological activity of the second polypeptide when compared to the activity of a monomer of the second polypeptide.

In one aspect, the invention encompasses a fusion polypeptide comprising a first polypeptide consisting of amino acids x to y of the polypeptide depicted in SEQ ID NO:10, wherein x is 110 to 154 and y is 201 to 270 fused to a second heterologous polypeptide, and wherein the fusion polypeptide is capable of forming a trimer.

30 In another aspect, the invention encompasses a fusion polypeptide comprising a first polypeptide consisting of amino acids x to y of the polypeptide depicted in SEQ ID

NO:10, wherein x is 110 to 173 and y is 179 to 270, fused to a heterologous polypeptide, and wherein the fusion polypeptide is capable of forming a trimer.

In another aspect, the invention encompasses a fusion polypeptide comprising a first polypeptide consisting of amino acids x to y of the polypeptide depicted in SEQ ID NO:10, wherein x is 110 to 154 and y is 201 to 270, with at least one substitution of an amino acid, wherein the first polypeptide is at least 80% identical to the polypeptide depicted in SEQ ID NO:10, and wherein said first polypeptide is fused to a second heterologous polypeptide, wherein the ability to trimerize is retained.

In another aspect, the invention encompasses a polypeptide domain comprising a first polypeptide consisting of amino acids x to y of the polypeptide depicted in SEQ ID NO:10, wherein x is 110 to 173 and y is 179 to 270, with at least one substitution of an amino acid, wherein the first polypeptide is at least 80% identical to the polypeptide depicted in SEQ ID NO:10, wherein the ability to trimerize is retained, and wherein the first polypeptide is fused to a second heterologous polypeptide.

In yet another aspect, the invention relates to a fusion polypeptide comprising amino acids 154 to 203 of the polypeptide depicted in SEQ ID NO:10, or alternatively, the invention relates to a fusion polypeptide comprising amino acids 120 to 180 of the polypeptide depicted in SEQ ID NO:10.

The invention further contemplates that the polypeptide capable of forming trimer, as described above and throughout the current specification, has at least three heptad repeats. However, it is understood that the trimerization polypeptide can have four heptad repeats, five heptad repeats, six heptad repeats, seven heptad repeats or more as desired by the ordinarily skilled artisan.

In still another aspect, the invention encompasses nucleic acids encoding the polypeptides of the invention. Methods of making recombinant vectors comprising the nucleic acids of the invention are also within the scope of the invention. In yet another aspect, the polypeptides encoded by the nucleic acid molecules of the invention are non-immunogenic in a subject to which they are administered.

In addition, it is contemplated that the nucleic acids of the invention can be transfected in host cells to produce recombinant polypeptides. In another aspect, the invention encompasses a method of producing a polypeptide encoded by the nucleic acid of the invention, comprising the steps of growing a host cell transfected with nucleic

acids of the invention in conditions favorable for expression of the polypeptide, and optionally isolating said polypeptide.

In yet another aspect, the invention encompasses a method of constructing a recombinant expression vector comprising the steps of linking a first nucleic acid
5 comprising nucleotides x to y of the nucleic acid depicted in SEQ ID NO:9, wherein x is nucleotides 331 to 460, and y nucleotides is 601 to 808, and a second nucleic acid encoding a heterologous polypeptide to the first nucleic acid into a compatible cloning site of an expression vector, followed by amplifying and isolating said recombinant expression vector.

10 In still another aspect, the invention encompasses a method of constructing a recombinant expression vector comprising the steps of linking a first nucleic acid comprising nucleotides x to y of the nucleic acid depicted in SEQ ID NO:9, wherein x is nucleotides 331 to 508, and y nucleotides is 546 to 808, and a second nucleic acid encoding a heterologous polypeptide to the first nucleic acid into a compatible cloning
15 site of an expression vector, followed by amplifying and isolating said recombinant expression vector.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1. Nucleic acids of the oligonucleotides utilized for construction of a
20 polynucleotide encoding a macrophage scavenger receptor-CD40L (TNFSF5) fusion polypeptide (sequence 34 is SEQ ID NO:1, sequence 33 is SEQ ID NO:2, sequence 32 is SEQ ID NO:3 and sequence 35 is SEQ ID NO:4).

Figures 2A-B. Figure 2A depicts the nucleic acid sequence (SEQ ID NO:5) encoding the IL2 secretion peptide fused to the scavenger receptor trimerization domain,
25 which is fused to a receptor binding domain of the CD40L (TNFSF5). The fusion polypeptide encoded by the nucleic acid is depicted as SEQ ID NO:6. Figure 2B depicts the nucleic acid sequence (SEQ ID NO:7)-encoding the human growth hormone secretion peptide fused to the scavenger receptor trimerization domain, which is fused to a receptor binding domain of the CD40L (TNFSF5). The fusion polypeptide-encoded by the nucleic
30 acid is depicted as (SEQ ID NO:8).

Figure 3 depicts a western blot demonstrating expression of the CD40L (TNFSF5) fusion polypeptide. Arrows indicate the location in the gel of the fusion polypeptide.

Lanes 1 and 2 are control using the isoleucine-CD40L (TNFSF5) trimerizing polypeptide, and lanes 3-6 are scavenger receptor trimerization domains fused to the receptor binding domain of CD40L (TNFSF5).

Figure 4. The coding region of the nucleic acid (SEQ ID NO:9) encoding the human macrophage scavenger receptor A is depicted including the encoded polypeptide (SEQ ID NO:10).

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates, in part, to a polypeptide domain capable of inducing trimers in heterologous polypeptides, fusion polypeptides comprising the trimer inducing domain, nucleic acids encoding the polypeptides, and methods of making and using the polypeptides.

In one embodiment, the invention relates to a portion of the extracellular domain of the macrophage scavenger receptor (SR-A) polypeptide corresponding to a coiled coil region, *i.e.*, the trimerization domain. The coiled coil region of the SR-A has trimerizing properties (Frank et al., 2000, J. Biol. Chem., 275:11672-11677). As such, this domain is useful in the present invention to provide a trimerizing domain, with no or reduced immunogenicity, fused to a heterologous polypeptide.

Thus, in one embodiment, the invention relates to a fusion polypeptide comprising a first polypeptide consisting of amino acids x to y of the polypeptide depicted in SEQ ID NO:10, wherein x is 110 to 154 and y is 201 to 270 fused to a second heterologous polypeptide. In another embodiment, the invention relates to a fusion polypeptide comprising a portion of the SR-A polypeptide consisting of amino acids x to y of the polypeptide depicted in SEQ ID NO:10, wherein x is 110 to 173 and y is 179 to 270 fused to a second heterologous polypeptide.

In a particular embodiment, the invention relates to a fusion polypeptide comprising amino acids 154 to 203 of the polypeptide depicted in SEQ ID NO:10 fused to a second heterologous polypeptide. In another particular embodiment, the invention relates to a fusion polypeptide comprising amino acids 120 to 180 of the polypeptide depicted in SEQ ID NO:10 fused to a second heterologous polypeptide.

In another embodiment, the invention relates to a fusion polypeptide comprising a first polypeptide consisting of amino acids x to y of the polypeptide depicted in SEQ ID

NO:10, wherein x is 110 to 154 and y is 201 to 270, with at least one substitution of an amino acid, wherein the first polypeptide is at least 80% identical to the polypeptide depicted in SEQ ID NO:10, wherein the ability to trimerize is retained, and wherein said first polypeptide is fused to a second heterologous polypeptide. And in yet another
5 embodiment, the invention relates to a fusion polypeptide comprising a first polypeptide consisting of amino acids x to y of the polypeptide depicted in SEQ ID NO:10, wherein x is 110 to 173 and y is 179 to 270, with at least one substitution of an amino acid, wherein the first polypeptide is at least 80% identical to the polypeptide depicted in SEQ ID
10 NO:10, wherein the ability to trimerize is retained, and wherein said first polypeptide is fused to a second heterologous polypeptide.

In another embodiment, the invention relates to a fusion polypeptide comprising a first polypeptide consisting of amino acids 154 to 203 of the polypeptide depicted in SEQ ID NO:10, with at least one substitution of an amino acid, wherein the first polypeptide is at least 80% identical to the polypeptide depicted in SEQ ID NO:10, wherein the ability
15 to trimerize is retained, and wherein said first polypeptide is fused to a second heterologous polypeptide. In yet another embodiment, the invention relates to a fusion polypeptide comprising a first polypeptide consisting of amino acids 154 to 203 of the polypeptide depicted in SEQ ID NO:10, wherein said first polypeptide is fused to a second heterologous polypeptide.

20 In the above embodiments, the second heterologous polypeptide preferably has a desired biological activity. A biological activity, as will be understood by one of ordinary skill, can be defined as binding of a ligand to a receptor, binding of a receptor to a ligand, binding affinity of a immunoglobulin-like polypeptide to its cognate antigen, or the like. Additionally, the biological activity may be an enzymatic activity.

25 In one embodiment, the fusion polypeptide the invention has reduced immunogenicity, and more preferably embodiment, the fusion polypeptide is non-immunogenic.

In one aspect, various species of macrophage scavenger receptor can serve as a trimerization motif for a fusion polypeptide, in particular, it is contemplated that bovine
30 (Genbank Accession No. X54183), murine (Genbank Accession No. L04275), human (Genbank Accession No. XM_005021), and/or simian scavenger receptor polypeptides can be used according to the teachings of the present invention. It is to be understood that

this is not an exhaustive listing and additional species' macrophage scavenger receptors are within the scope of the invention.

A consensus coiled coil polypeptide sequence, as it pertains to the invention, follows the basic pattern as set forth in Burkhard et al. (2001, Trends Cell Biol., 11:82).

- 5 The positions of amino acids in the heptad repeats are typically designated alphabetically as 'a.b.c.d.e.f.g.' Positions a and d are apolar residues and there is a strong preference for specific amino acids at these positions. An apolar residue is understood by one of skill in the art to include alanine, isoleucine, glycine, proline, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine. Particular residues at each of these positions can
- 10 determine the nature of the secondary and tertiary structure of the coiled-coil *i.e.*, dimeric versus trimeric. Indeed, targeted mutation has been performed to redesign dimeric leucine zippers into the trimeric isoleucine zippers by mutation of residues in positions a and d (U.S. Patent No. 5,716,805).

- Computer programs can be enlisted to assist in the identification of coiled coil
- 15 polypeptides useful in the present invention. For example, FOLDER has been used to identify particular domains based on sequence identity (Srinivasan et al., (1993) Prot. Sci., 2:277-289) and can be used to identify or modify a coiled domain as used herein. Amino acids of CD40L (TNFSF5) were analyzed using folder program to study and predict trimerization propensity of several different heptad repeats within the CD40L
- 20 (TNFSF5) coiled coil domains. Further, the multicoil program (Harbury et al., (1993) Science, 262:1401) can be used to confirm that a sequence, *i.e.*, a scavenger receptor sequence such as human, murine and bovine polypeptides, are coiled coils. This program finds particular utility when a native sequence is mutated to alter the properties of a polypeptide, such as, for example to increase trimerization and/or decrease
- 25 immunogenicity. Thus a mutant trimerizing polypeptide can be analyzed by computer program to determine whether it retains required characteristics.

- In one embodiment, a trimerization polypeptide of the invention has at least 80% sequence identity (at least 85%, at least 90%, at least 95%, at least 97.5%, or at least 99%, and/or at least 99.5%) with a naturally occurring trimerizing polypeptide, *e.g.*, such as the
- 30 polypeptide depicted in SEQ ID NO:10, such sequence identity being determined by comparing the amino acids of the polypeptides when aligned so as to maximize overlap and identity while minimizing sequence gaps. The percent identity of two amino acids or

two nucleic acids can be determined by visual inspection and mathematical calculation, or more preferably, the comparison is done by comparing sequence information using a computer program. An exemplary, preferred computer program is the Genetics Computer Group (GCG; Madison, WI) Wisconsin package version 10.0 program, 'GAP' (Devereux et al., (1984) Nucl. Acids Res. 12: 387). The preferred default parameters for the 'GAP' program includes: (1) The GCG implementation of a unary comparison matrix (containing a value of 1 for identities and 0 for non-identities) for nucleotides, and the weighted amino acid comparison matrix of Gribskov and Burgess, Nucl. Acids Res. 14:6745, (1986) as described by Schwartz and Dayhoff, eds., (1979) Atlas of Polypeptide Sequence and Structure, National Biomedical Research Foundation, pp. 353-358; or other comparable comparison matrices; (2) a penalty of 30 for each gap and an additional penalty of 1 for each symbol in each gap for amino acid sequences, or penalty of 50 for each gap and an additional penalty of 3 for each symbol in each gap for nucleotide sequences; (3) no penalty for end gaps; and (4) no maximum penalty for long gaps.

In one embodiment, a trimerization polypeptide of the invention comprises a sequence having at least 80% identity with amino acids x to y of the polypeptide depicted in SEQ ID NO:10, wherein x is 110 to 154 and y is 201 to 270. In another embodiment, the trimerization polypeptide of the invention comprises a sequence having at least 80% identity with amino acids x to y of the polypeptide depicted in SEQ ID NO:10, wherein x is 110 to 173 and y is 179 to 270.

Other programs used by those skilled in the art of sequence comparison can also be used, such as, for example, the BLASTN program version 2.0.9, available for use via the National Library of Medicine website www.ncbi.nlm.nih.gov/gorf/wblast2.cgi, or the UW-BLAST 2.0 algorithm. Standard default parameter settings for UW-BLAST 2.0 are described at the following Internet site: sapiens.wustl.edu/blast/blast/#Features. In addition, the BLAST algorithm uses the BLOSUM62 amino acid scoring matrix, and optional parameters that can be used are as follows: (A) inclusion of a filter to mask segments of the query sequence that have low compositional complexity (as determined by the SEG program of Wootton and Federhen (Computers and Chemistry, 1993); also see Wootton and Federhen, 1996, Analysis of compositionally biased regions in sequence databases, Methods Enzymol. 266: 554-71) or segments consisting of short-periodicity internal repeats (as determined by the XNU program of Claverie and States (Computers

and Chemistry, 1993)), and (B) a statistical significance threshold for reporting matches against database sequences, or E-score (the expected probability of matches being found merely by chance, according to the stochastic model of Karlin and Altschul (1990); if the statistical significance ascribed to a match is greater than this E-score threshold, the match will not be reported.); preferred E-score threshold values are 0.5, or in order of increasing preference, 0.25, 0.1, 0.05, 0.01, 0.001, 0.0001, 1e-5, 1e-10, 1e-15, 1e-20, 1e-25, 1e-30, 1e-40, 1e-50, 1e-75, or 1e-100.

A trimerization domain can be fused to a heterologous polypeptide at the amino terminus, carboxyl terminus and/or or both. In addition, the trimerization domain can be internal to the heterologous polypeptide. Regardless of the location of the fusion of the trimerization domain and the heterologous polypeptide, it is to be understood that the trimerizing domain retains its ability to induce trimers (homotrimers and/or heterotrimers), and the heterologous polypeptide also retains its biological activity.

As used herein, the term "fusion polypeptide" refers to a contiguous polypeptide expressed from a single nucleic acid molecule, wherein the nucleic acid encodes in the same reading frame either two or more heterologous polypeptides, or domains of a polypeptide arranged in a nonnaturally occurring order. In addition to the polypeptides of the invention, other examples of fusion polypeptides include an Fc domain of an antibody or a leucine zipper domain fused to a soluble extracellular domain of a transmembrane polypeptide, *e.g.*, CD40L (TNFSF5). WO 93/08207 and WO 96/40918 describe the preparation of oligomeric soluble forms of CD40L (TNFSF5), including an immunoglobulin fusion polypeptide and a mutated leucine zipper fusion polypeptide, respectively; the methods of constructing and assaying the products discussed therein are also applicable to the compositions of the present invention. The fusion of a trimerizing domain to a heterologous polypeptide can also occur through a linker, such as an epitope tag, or a flexible linker that assists in structural formation.

It is to be understood that any portion of a polypeptide having a desired biological activity can be expressed as a fusion to the multimerizing polypeptides of the invention. Examples include but are not limited to transmembrane polypeptides, and non-transmembrane polypeptides such as enzymes, hormones, and cytokines. In particular, it is contemplated that fusion polypeptides of GM-CSF, IL3, GM-CSF and IL3, M-CSF,

and members of the hematopoietin receptor family (Cosman, et al., (1990) Trends Biochem. Sci., 15:265). Homo and hetero oligomers are further contemplated.

Additional examples of heterologous polypeptides that can be trimerized include, but are not limited to the tumor necrosis factor super family, which are listed below.

- 5 Each pairing is listed first by the ligand followed by the corresponding receptor where a ligand may have more than one receptor. The nomenclature for the TNF family member symbols is as described in Locksley et al. (2001, Cell, 104:487:501), wherein a ligand is denoted by tumor necrosis factor super family (TNFSF) and receptors are denoted by tumor necrosis factor receptor super family (TNFRSF) in numerical order.
- 10 The symbol for the TNF family member ligand or receptor is first, followed by a Genbank Accession number in double parenthesis, which is followed by the various common names for the family member as applicable: TNFSF1 ((Genbank Accession No. X01393), TNFB, Lymphotoxin (LT)-alpha) and its receptor tumor necrosis factor receptor TNFRSF1A ((Genbank Accession No. M75866), TNF-R); TNFSF2 ((Genbank
15 Accession No. X02910) tumor necrosis factor (TNF)) and its receptor TNFRSF1B ((Genbank Accession No. M32315) TNF-R); TNFSF3 ((Genbank Accession No. L11016) LT-beta) and its receptor TNFRSF3 ((Genbank Accession No. L04270) LTBR, TNFR2-RP, CD18, TNFR-RP, TNFCR, TNF-R-III); TNFSF4 ((Genbank Accession No. D90224) OX-40L) and its receptor TNFRSF4 ((Genbank Accession
20 No. X75962) OX40); TNFSF5 ((Genbank Accession No. X67878) CD40L) and its receptor TNFRSF5 ((Genbank Accession No. X60592) CD40); TNFSF6 ((Genbank Accession No. U11821) FasL) and its receptors TNFRSF6 (Genbank Accession No. M67454) FAS, CD95) and TNFRSF6B ((Genbank Accession No. AF104419) DcR3); TNFSF7 ((Genbank Accession No. L08096) CD70, CD27L) and its receptor
25 TNFRSF7 ((Genbank Accession No. M63928) CD27); TNFSF8 ((Genbank Accession No. L09753) CD30LG) and its receptor TNFRSF8 ((Genbank Accession No. M83554) CD30); TNFSF9 ((Genbank Accession No. U03398) 4-1BB-L) and its receptor TNFRSF9 ((Genbank Accession No. L12964) 4-1BB); TNFSF10 ((Genbank Accession No. U37518) TRAIL) and its receptors TNFRSF10A ((Genbank Accession No. U90875) DR4), TNFRSF10B ((Genbank Accession No. AF012628) DR5, KILLER, TRAIL-R2), TNFRSF10C ((Genbank Accession No. AF012536) DcR1, TRAILR3), and TNFRSF10D ((Genbank Accession No. AF029761) DcR2, TRAILR4); TNFSF11 ((Genbank

Accession No. AF013171) RANKL, OPGL) and its receptors TNFRSF11A ((Genbank
Accession No. AF018253 RANK) and TNFRSF11B (Genbank Accession No. U94332)
OPG); TNFSF12 ((Genbank Accession No. AF030099) TWEAK) and its receptors
TNFRSF12 ((Genbank Accession No. U72763) DR3, TRAMP) and TNFRSF12L
5 (DR3L); TNFSF13 ((Genbank Accession No. NM_003808) APRIL) and its receptors
TNFRSF13 and TNFSF13B ((Genbank Accession No. AF136293) THANK, BLYS);
TNFSF14 ((Genbank Accession No. AF036581) LIGHT, LT-gamma, HVEM-L) and its
receptor TNFRSF14 ((Genbank Accession No. U70321) HVEM, ATAR, TR2, LIGHTR,
HVEA); TNFSF15 ((Genbank Accession No. AF039390) TL1, VEGI) and its receptor
10 TNFRSF15; TNFSF16 and its receptor TNFRSF16 ((Genbank Accession No. M14764)
nerve growth factor receptor (NGFR), p75NTR); TNFSF17 and its receptor TNFRSF17
((Genbank Accession No. Z29574) BCMA); TNFSF18 ((Genbank Accession
No. AF125303) AITRL TL6 hGITRL) and its receptor TNFRSF18 ((Genbank Accession
No. AF125304) AITR, GITR); TNFSF19 and its receptor TNFRSF19 (Genbank
15 Accession No. AF173166); TNFSF20 and its receptor TNFRSF20 (Locksley et al., 2001,
Cell, 104:487:501). In a specific embodiment, a polypeptide that can be utilized as a
heterologous polypeptide by the methods and compositions of the invention is CD40L
(TNFSF5).

Additional polypeptides can be fused to the fusion polypeptide to facilitate
20 purification such as, for example, epitope tags can be placed on the amino terminus,
carboxyl terminus or internally. Well known epitope tags include, *e.g.*, histidine tag,
FLAG tag, and GST among others. It is further contemplated that an epitope tag can be
genetically engineered to include a proteolytic cleavage site between the tag and the rest
of the polypeptide such that the epitope tag can be cleaved and removed from the fusion
25 polypeptide. Examples of proteolytic enzymes known to recognize unique amino acids in
polypeptides include Factor Xa and thrombin and are commercially available.

The invention contemplates higher order multimers of a fusion polypeptide of the
invention, such that it has increased activity relative to a non-multimerized polypeptide.
As used herein, a "multimer" or a "multimerized polypeptide" can include a dimer,
30 trimer, tetramer, dimer of a trimer, trimer of a trimer, dimer of a tetramer, trimer of a
tetramer and so on. Examples of additional multimerization domains can include leucine
zippers or Fc domains. Accordingly, a polypeptide comprising a dimerizing domain,

(e.g., an Fc domain) and trimerizing domain (e.g., a scavenger receptor alpha trimerization domain) can induce a dimer of a trimer such that six polypeptides associate. It is further contemplated that these multimers can have bioactivity that is greater than monomers or lower order multimers. For example, it is contemplated that a trimer will
5 have higher activity than a dimer.

The terms "reduced immunogenicity" or "non-immunogenic" are understood to mean that a therapeutic, such as a polypeptide of the invention, fails to induce an appreciable immune response in a subject, preferably a human, to which it has been administered. An appreciable immune response is one that is measurable such that one of
10 ordinary skill in the art would recognize it as having been induced by the polypeptide of the invention. This definition excludes normal variations due to differences in reagents used in the assays, variability in the samples, or where there are otherwise imperfections in the testing procedures. An immune response can be measured by any variety of standard techniques, such as for example using an enzyme linked immunosorbent assay
15 (ELISA) to detect antibodies made in response to a therapeutic, by measuring cytokine production by the subject in appropriate time periods subsequent to administration of the polypeptide, and/or by measuring proliferation of particular immune cell types, among other well known methods.

One exemplary method of reducing or eliminating immunogenicity in a
20 therapeutic polypeptide, is to use polypeptides derived from the same species as will be treated. For example, when a polypeptide is to be administered to a human, the polypeptide should be human derived. If in this example human polypeptides are not available, the polypeptides can be humanized such that immunogenicity is reduced. Humanizing a polypeptide can be performed, for example, in a manner analogous to
25 humanizing antibodies, wherein a mouse antibody sequence is humanized by mutating individual amino acids to correspond to human polypeptide sequences such that the antibody does not induce a strong human anti-mouse response when administered.

Nucleic acids encoding the foregoing polypeptides are also within the scope of the invention. These nucleic acids can be cloned and mutated by well known techniques and
30 are commonly propagated in plasmids or other vectors (Sambrook et al. eds., (1989) Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY). Vectors can also include

additional components to facilitate replication in prokaryotic and/or eukaryotic cells, integration of the construct into a eukaryotic chromosome, and markers to aid in selection of and/or screening for cells containing the construct. Vectors of the invention are recombinant DNA vectors including, but not limited to, plasmids, phages, phagemids, cosmids, viruses, retroviruses, and the like, which are used to insert heterologous polynucleotides into a cell.

As used herein, the term "linking" is understood to mean that two molecules are brought into proximity to one another by either an affinity interaction and/or a covalent attachment such as for example, ligation of nucleotides to one another in cloning, by chemical cross-linking, or by some other similar method of linking. Nucleotides can be obtained from genomic, cDNA or vector sources and linked to heterologous nucleic acids by traditional digestion and ligation in cloning, or by amplification of desired portions of sequences during PCR, among other methods.

In one embodiment, a nucleic acid molecule encoding a fusion polypeptide of the invention is operably linked to a heterologous control element. A nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid. Operably linked can also mean that two different nucleic acid molecules encoding different polypeptides have transcription induced simultaneously. In the case of operably linked nucleic acids, they can also be contiguous in a single transcriptional unit, while translation is directed from one or more ribosomal start sites. In addition, operably linked can mean that two polypeptide coding regions can be joined in frame such that they encode a single contiguous polypeptide, *i.e.*, a fusion polypeptide.

An enhancer or silencer is operably linked to a nucleic acid when it is positioned relative to the nucleic acid in such a manner as to increase or decrease the transcription of the nucleic acid. Enhancers and silencers can be located upstream, downstream or embedded within the coding regions of the nucleic acid.

Expression vectors can be for expression of a fusion polypeptide of the invention in prokaryotic (*e.g.*, *E. coli*) or eukaryotic cells (*e.g.*, insect cells (using baculovirus expression vectors), yeast cells, plant cells, or mammalian cells). Suitable host cells include prokaryotic, eukaryotic and plant cells, and are discussed further in Goeddel, (1990) Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, CA. Expression of proteins in prokaryotes is most often carried out in *E. coli* with vectors containing constitutive or inducible promoters directing the protein expression.

Examples of suitable inducible *E. coli* expression vectors include pTrc (Amann et al., (1988) Gene 69:301-315) and pET 11d (Studier et al., (1990) Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, California 60-89).

5 In another embodiment, the expression vector is a yeast expression vector. Examples of vectors for expression in yeast *S. cerevisiae* include pYepSec1 (Baldari et al., (1987) EMBO J. 6:229-234), pMFa (Kurjan and Herskowitz, (1982) Cell 30:933-943), pJRY88 (Schultz et al. (1987) Gene 54:113-123), pYES2 (Invitrogen Corporation, San Diego, CA), and pPicZ (Invitrogen Corp, San Diego, CA). Alternatively, the
10 expression vector is a baculovirus expression vector such as, for example, the pAc series (Smith et al. (1983) Mol. Cell Biol. 3:2156-2165). In another embodiment, a nucleic acid of the invention is expressed in mammalian cells using a mammalian expression vector. Examples of mammalian expression vectors are numerous and include but are not limited to pCDM8 (Seed (1987) Nature 329:840) and pMT2PC (Kaufman et al., (1987) EMBO J.
15 6:187-195).

 A recombinant mammalian expression vector can also be designed such that it is capable of directing expression of the nucleic acid preferentially in a particular cell type (e.g., tissue-specific regulatory elements are used to express the nucleic acid). Tissue-specific regulatory elements are known in the art. Non-limiting examples of suitable
20 tissue-specific promoters include the albumin promoter (liver-specific; Pinkert et al., (1987) Genes Dev. 1:268-277), lymphoid-specific promoters (Calame and Eaton (1988) Adv. Immunol. 43:235-275), in particular promoters of T cell receptors (Winoto and Baltimore (1989) EMBO J. 8:729-733), immunoglobulins (Banerji et al. (1983) Cell 33:729-740; Queen and Baltimore (1983) Cell 33:741-748), and mammary gland-specific
25 promoters (e.g., milk whey promoter; U.S. Patent No. 4,873,316 and European Application Publication No. 264,166).

 Another aspect of the invention pertains to host cells into which a recombinant expression vector of the invention has been introduced. The terms "host cell" and "recombinant host cell" are used interchangeably herein. It is understood that such terms
30 refer not only to the particular subject cell but to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the

parent cell, but are still included within the scope of the term as used herein. A host cell can be any prokaryotic (*e.g.*, *E. coli*), eukaryotic cell (*e.g.*, insect cells, yeast or mammalian cells) or plant cells.

Cells used in the invention can be genetically engineered to express a fusion polypeptide of the invention. By genetically engineered is meant that the cell has been transfected, transformed or transduced with a recombinant polynucleotide molecule, and/or otherwise altered (*e.g.*, by homologous recombination and gene activation) so as to cause the cell to express the fusion polypeptide. Methods and vectors for genetically engineering cells and/or cell lines are well known to those of skill in the art; for example, various techniques are illustrated in *Current Protocols in Molecular Biology*, Ausubel et al., eds. (Wiley & Sons, New York, (1988) and quarterly updates) and Sambrook et al., (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Laboratory Press).

It is further contemplated that the fusion polypeptides of the invention can be expressed in transgenic animals, such as for example, in mice, goats or cows. The latter example, the expression of the fusion polypeptide may be designed such that it is secreted into the animals milk. A transgenic animal can be produced by introducing the nucleic acid encoding a fusion polypeptide of the invention into a fertilized oocyte or an embryonic stem cell. Such host cells can then be used to create non-human transgenic animals.

Vector DNA can be introduced into cells via conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, or electroporation. Suitable methods for transforming or transfecting host cells can be found in Sambrook, et al. (*supra*), and other laboratory manuals. For stable transfection of mammalian cells, it is known that, depending upon the expression vector and transfection technique used, only a small fraction of cells may integrate the transfected DNA into their genome. In order to identify and select these integrants, a gene that encodes a selectable marker (*e.g.*, for resistance to antibiotics) is generally introduced into the host cells along with the gene of interest. Preferred selectable markers include those which confer resistance to drugs,

such as G418, hygromycin and methotrexate. Cells stably transfected with the introduced nucleic acid can be identified by drug selection.

In another embodiment, fusion of a trimerization polypeptide of the invention to a second heterologous polypeptide that is a ligand can result in enhanced signaling through the ligand's receptor. While not meant to be limiting, the mechanism of this increase can occur by increased binding and activation of the receptor such that the receptor has increased biological activity. For example multimerized CD40L (TNFSF5) has been shown, when administered to a cell population, to enhance immune cell proliferation relative to untreated cells (see Example, below) and also to cells that are treated with non-multimerized CD40L (TNFSF5), thus according to the invention, when soluble CD40L (TNFSF5) is fused to a trimerization polypeptide of the invention domain it has increased activity relative to soluble CD40L (TNFSF5) that is not trimerized.

The terms "enhanced" or "increased" are understood to include any measurable elevation in biological activity as measured against an untreated control. An increase can be 10%, 20%, 30%, 40% or more and increases can exceed 100% of the activity of controls. Examples of assays to measure increases include but are not limited to evaluating cytokine release, induction of surface molecule expression, increased or decreased cellular proliferation, increased or decreased apoptotic cell death, and biochemical activation, *e.g.*, phosphorylation patterns. In a particular example, addition of trimerized CD40L (TNFSF5) to cells can result in inhibition of IL-4 induced IgE secretion or elevated expression of CD23 in mixed lymphocyte cultures. In a particular embodiment of the invention, multimers of trimers of the invention have activity that is higher than the activity of the non-multimerized trimers.

In yet another embodiment, fusion of a multimerization polypeptide, such as a trimerization domain, to a second heterologous polypeptide that is a receptor can result in decreased signaling through the corresponding, native receptor. While not meant to be limiting, the mechanism of this reduction can occur by competitive binding of the soluble polypeptide with the receptor's ligand, wherein this binding prevents the ligand from activating the receptor. For example, administration of TNFR:Fc (*i.e.*, Etanercept), a dimer, results in a reduction in the signaling through the TNF-alpha receptor relative to untreated cells as measured, in part, by reduced disease symptoms (Spencer-Green, 2000,

Ann. Rheum. Dis., 59 Suppl 1, pp. 46-9). Thus, a multimer of a TNFR according to the invention can inhibit TNF-alpha mediated inflammation.

The term "reduced" is understood to include any measurable decrease in biological activity as measured against an untreated control. More particularly, a decrease can be 10%, 20% or more including up to an 100% reduction. Examples of assays to measure this reduction include but are not limited to increased or decreased cytokine release, induction of surface molecule expression, increased or decreased cellular proliferation, increased or decreased apoptotic cell death, and biochemical activation, *e.g.*, phosphorylation patterns.

The activity of the fusion polypeptide is partly determined by its affinity to a binding partner (*e.g.*, receptor or ligand). The affinity of the trimerized molecules for their respective binding partner can be measured using biospecific interaction analysis (BIA), which is a biosensor, *i.e.*, an instrument that combines a biological recognition mechanism with a sensing device or transducer. An exemplary biosensor is BIOcoreTM (Pharmacia). This uses the optical phenomenon surface plasmon resonance to monitor the interaction between two biological molecules. Molecule pairs having affinity constants in the range of 10^5 to 10^{10} M^{-1} , and association rate constants in the range of 10^3 to $10^6 \text{ M}^{-1} \text{ s}^{-1}$ are suited for this method.

It is also contemplated that, as trimers (or higher order multimers) have more binding sites for their binding partner than dimers or monomers, the multimerized polypeptides of the invention will also have higher avidity for binding partners than will monomers or dimers (or lower order multimers). This higher degree of avidity increases the specific binding of the multimers to their binding partners. As such, in one embodiment, it is contemplated that the polypeptides of the invention can be linked to heterologous therapeutic molecules for high efficiency delivery to target cells expressing a binding partner. Examples of heterologous molecules that can be linked include, but are not limited to, radioactive moieties, *e.g.*, I^{125} , Y^{90} , and/or polypeptide toxins, *e.g.*, ricin, botulism toxin. The linkage can be through standard cross linking techniques well known in the art.

Pharmaceutical Preparations and Methods of Administration

The polypeptides of the invention can be formulated as compounds suitable for administration to a patient in need of treatment. Such compounds are useful in treating immune related disorders and/or in increasing the stimulation of an otherwise normal immune response. A therapeutically effective dose refers to that amount of the compound sufficient to result in amelioration of symptoms of, for example, immunodeficiency or inflammatory conditions or diseases. Furthermore, when a polypeptide of the invention is co-administered with another therapeutic agent, doses are modified according to any interactions that may occur between the therapeutic agents.

Effective Dose

Toxicity and therapeutic efficacy of compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD50 (the dose lethal to 50% of the population) and the ED50 (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD50 /ED50. Compounds which exhibit large therapeutic indices are preferred.

While compounds that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such compounds to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC50 (*i.e.*, the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture prior to administration to a patient.

When another therapeutic is administered in combination with the polypeptides of the invention (coadministration), the polypeptides of the invention can be delivered either

prior to, simultaneous with, or after delivery of the second therapeutic. Simultaneous administration encompasses mixing the polypeptides of the invention with the second therapeutic prior to administration to the patient, or administration to the patient in separate infusions, albeit at the same time. It is also contemplated that the dose of the second therapeutic should be consistent with established therapeutic ranges, however, should there can be an increase in effectiveness of the therapeutic when used in combination with a polypeptides of the invention that is greater than the sum of either alone there is synergy. Thus, in the case of synergy, it will be understood that doses can be decreased relative to recommended ranges in light of enhanced effectiveness.

10 In one embodiment of the invention, a polypeptide of the invention is administered one time per week to treat the various medical disorders disclosed herein, in another embodiment is administered at least two times per week, and in another embodiment is administered at least once per day. An adult patient is a person who is 18 years of age or older. If injected, the effective amount, per adult dose, of a polypeptide of the invention ranges from about 1-500 mg/m², or from about 1-200 mg/m², or from about 15 1-40 mg/m² or about 5-25 mg/m². Alternatively, a flat dose may be administered, whose amount may range from 2-500 mg/dose, 2-100 mg/dose or from about 10-80 mg/dose. If the dose is to be administered more than one time per week, an exemplary dose range is the same as the foregoing described dose ranges or lower. Preferably, polypeptides of the invention are administered two or more times per week at a per dose range of 20 25-100 mg/dose.

In one embodiment of the invention, the various indications described below are treated by administering a preparation acceptable for injection containing a polypeptide of the invention at 80-100 mg/dose, or alternatively, containing 80 mg per dose. The dose 25 can be administered at biweekly, weekly doses, or separated by several weeks (for example 2 to 8).

If a route of administration of the polypeptides of the invention other than injection is used, the dose is appropriately adjusted in accord with standard medical practices. For example, if the route of administration is inhalation, dosing may be one to 30 seven times per week at dose ranges from 10 mg/dose to 50 mg per dose.

In many instances, an improvement in a patient's condition will be obtained by injecting a dose of up to about 100 mg of the polypeptides of the invention one to three

times per week over a period of at least three weeks, though treatment for longer periods may be necessary to induce the desired degree of improvement. For incurable chronic conditions, for example, patients with bone marrow failure caused by a genetic disorder, the regimen may be continued indefinitely.

- 5 For pediatric patients (ages 4-17), a suitable regimen involves the subcutaneous injection of 0.4 mg/kg to 5 mg/kg of a the polypeptides of the invention, administered by subcutaneous injection one or more times per week.

Formulations and Use

- 10 Pharmaceutical compositions for use of the compositions of the present invention may be formulated in conventional manner using one or more physiologically acceptable carriers or excipients. Thus, the compounds and their physiologically acceptable salts and solvates may be formulated for administration by inhalation or insufflation (either through the mouth or the nose) or buccal, parenteral or rectal administration.

- 15 For buccal administration the compositions may take the form of tablets or lozenges formulated in conventional manner.

- The compounds may be formulated for parenteral administration by injection, *e.g.*, by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, *e.g.*, in ampoules or in multi-dose containers, with an added
20 preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, *e.g.*, sterile pyrogen-free water, before use.

- 25 The compounds may also be formulated in rectal compositions such as suppositories or retention enemas, *e.g.*, containing conventional suppository bases such as cocoa butter or other glycerides.

- In addition to the formulations described previously, the compounds may also be formulated for injection. Such formulations are typically stored as aqueous solutions that
30 can be frozen and thawed, but can be also be prepared as lyophilized samples for extended storage. These formulations are frequently presented in a solution that is isotonic with human blood, namely, about 150 mM and buffered. Common buffers

include sodium phosphate and sodium acetate and the pH range can be from 1 to 14, but is more typically around 4.5 to 6. These formulations also can have a polyol (*e.g.*, sorbitol, trehalose, sucrose, etc.) and a surfactant (*e.g.*, polysorbate, Tween 80, etc.) and do not require sodium chloride to isotonic in solution.

5 The polypeptides of the invention can also be formulated as a depot preparation. Such long acting formulations may be administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the compounds may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly
10 soluble derivatives, for example, as a sparingly soluble salt.

 The compositions may, if desired, be presented in a pack or dispenser device which may contain one or more unit dosage forms containing the active ingredient. The pack may for example comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration.

15

EXAMPLES

Cloning

 Constructs were made by linking three different nucleic acids: 1) the receptor binding domain (RBD) of human CD40L (TNFSF5) as a 0.4kb Xba1-Not1 previously
20 fused to a CD40L (TNFSF5)194W deletion as described in Morris et al. (1999, J. Biol. Chem., 274:418-423); 2) the scavenger receptor trimerization sequence AvrII -Xba1 fragment generated as described below; 3) and the 5.6 kb Spe1- Not1 fragment consisting of the pSMAG expression vector containing the signal sequences and the transcriptional promoter. The resulting nucleic acids are depicted in SEQ ID NOs:5 and 7.

25 The AvrII -Xba1 fragment consisting of the 7 heptad repeat from the scavenger receptor coding sequence were generated through hybridization and ligation of two sets of synthetic oligonucleotides shown in Figure 1 (SR oligo sequences). The oligonucleotide sequence was designed to contain AvrII restriction site overhang at the 5' end and the Xba I restriction site overhang (compatible sequence) at the 3' end of the synthetic
30 scavenger receptor fragment. The XbaI site is TCTAGA, the AvrII restriction site is C/CTAGG and the SpeI restriction site is A/CTAGT. Ligation of Spe1 to AvrII generates

the sequence ACTAGG encoding amino acids threonine and arginine respectively (single amino acid letter code T and R).

Oligos 33 (SEQ ID NO:2) and 35 (SEQ ID NO:4) were phosphorylated using T4 polynucleotide kinase and ATP at 37°C for 1 hour. The appropriate oligonucleotide
5 preparations were mixed together in two separate reaction vessels (eppendorf tubes) at 2 µg/ml concentration and annealed by heating each oligonucleotide pair to 95°C for 2 minutes and then held at 65°C for 3 minutes and allowed to cool gradually to room temperature (25°C) over 45 minutes. Oligo 32 (SEQ ID NO:3) was annealed to
10 complementary oligo 35 and Oligo 33 was annealed to complementary oligo 34 (SEQ ID NO:1). The two sets of reactions were mixed together and ligated for 1 hour using DNA Ligase. The ligation reaction was analyzed using preparative 3% Nusieve® agarose gel electrophoresis and the approximately 150 basepairs fragment corresponding to the scavenger receptor AvrII/XbaI fragment was visualized and isolated from the gel.

These nucleic acids were cloned upstream (*i.e.*, N-terminal end) of the receptor
15 binding domain (RBD) of CD40L (TNFSF5) by ligating to an XbaI/NotI fragment containing the CD40L (TNFSF5) RBD and a NotI/SpeI fragment containing the pSMAG vector and the signal sequence. These scavenger receptor nucleic acids replaced the nucleic acids encoding the mutein leucine zipper that were previously used to trimerize/stabilize the trimers of soluble CD40L (TNFSF5). The expression vector pSMAG used
20 for these constructs contained the transcriptional promoter and either the IL2 signal sequence or the human growth hormone (GH) signal sequence to enable secretion of CD40L (TNFSF5).

The nucleic acid of the final constructs was analyzed on an automated sequencer. Sequence from the signal sequence through the junction of the RBD of CD40L (TNFSF5)
25 was obtained to confirm correct ligation. Figure 2A depicts a fusion polypeptide of the invention where amino acids 1 to 24 are the IL2 signal peptide, amino acids 25 to 39 are linker sequences, amino acids 40 to 88 are the trimerization domain, amino acids 89 to 95 are linker sequences and amino acids 96 to 244 are the CD40L (TNFSF5) 194W deletion sequences and Figure 2B depicts a fusion polypeptide of the invention where amino acids
30 1 to 25 are the IL2 signal peptide, amino acids 26 to 29 are linker sequences, amino acids 30 to 78 are the trimerization domain, amino acids 79 to 85 are linker sequences and amino acids 86 to 234 are the CD40L (TNFSF5) 194W deletion sequences.

Expression

COS cells were transfected with plasmid DNA with 2 µg in each well in a 6 well transfection vessel and the growth / conditioned media was harvested after 3 days and analyzed on SDS PAGE western blots using a polyclonal anti CD40L (TNFSF5) antibody. The resulting western blot, shown in Figure 3, demonstrates that both isoleucine zipper CD40L (TNFSF5) (Morris et al., (1999) J. Biol. Chem., 274:418-423) and the two forms of the scavenger receptor (SR)-CD40L (TNFSF5), described above, are expressed and secreted. Lanes 1, 3 and 5 contain harvest supernatant at 1:10 dilution and lanes 2, 4 and 6 contain supernatant at 1:30 dilution. Lanes contain harvest supernatant from cells transfected as follows: lanes 1 and 2 contain isoleucine zipper CD40L (TNFSF5) cDNA, lanes 4 and 6 contain growth hormone signal sequence linked to the scavenger receptor trimerization domain-CD40L (TNFSF5) (GH-SR-CD40L), lanes 5 and 6 contain IL2 signal sequence linked to the scavenger receptor trimerization domain-CD40L (TNFSF5) (IL2-SR-CD40L).

Bioactivity

The transfection harvest media was analyzed for bioactivity in a B cell proliferation bioassay as described by Morris et al. (1999, J. Biol. Chem., 274:418-423). Costimulation of human blood B cell proliferation was assessed by culturing T-cell depleted PBMC (E) in round bottom 96 well microtiter plates (1×10^5 cells in 200 µl) for 4 days in the presence of a titration of huCD40L (TNFSF5). Cells were pulsed with tritiated thymidine (91 µCi/well) for the final 18 hr culture period. Cells cultured with 1 µg/ml of isoleucine zipper CD40L (TNFSF5) were included as a positive control. Control cells were included as negative control. B cells are the only cells in the E prep that will proliferate in response to CD40L (TNFSF5). The results demonstrate that transfection supernatants containing the scavenger receptor trimerization domain fused to RBD of CD40L (TNFSF5), with the growth hormone signal sequence, are about as active as the leucine zipper form of the CD40L (TNFSF5).

Equivalents and References

The present invention is not to be limited in scope by the specific embodiments described herein, which are intended as single illustrations of individual aspects of the invention, and functionally equivalent methods and components are within the scope of

the invention. Indeed, various modifications of the invention, in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and accompanying drawings. Such modifications are intended to fall within the scope of the appended claims.

- 5 All publications, patents and patent applications mentioned in this specification are herein incorporated by reference into the specification to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated herein by reference.

CLAIMS

What is claimed is:

1. A fusion polypeptide comprising a first polypeptide consisting of amino acids x to y of the polypeptide depicted in SEQ ID NO:10, wherein x is 110 to 173 and y is 179 to 270, fused to a second heterologous polypeptide, and wherein the fusion polypeptide is capable of forming a trimer.
2. A fusion polypeptide comprising a first polypeptide consisting of amino acids x to y of the polypeptide depicted in SEQ ID NO:10, wherein x is 110 to 173 and y is 179 to 270 with at least one substitution of an amino acid, wherein the first polypeptide is at least 80% identical to the polypeptide depicted in SEQ ID NO:10 and the ability to trimerize is retained, and wherein said first polypeptide is fused to a second heterologous polypeptide.
3. The fusion polypeptide of claim 1, wherein the first polypeptide consists of amino acids 154 to 203 of the polypeptide depicted in SEQ ID NO:10.
4. The fusion polypeptide of claim 1, 2 or 3, wherein the second polypeptide is a soluble portion of a tumor necrosis factor receptor super family (TNFRSF).
5. The fusion polypeptide of claim 4, wherein the second polypeptide is a soluble portion of a tumor necrosis factor super family (TNFSF) ligand.
6. The fusion polypeptide of claim 5, wherein the second polypeptide is a soluble portion of a polypeptide selected from the group consisting of TNFRSF1A, TNFRSF1B, TNFRSF3, TNFRSF4, TNFRSF5, TNFRSF6, TNFRSF6B, TNFRSF7, TNFRSF8, TNFRSF9, TNFRSF10A, TNFRSF10B, TNFRSF10C, TNFRSF10D, TNFRSF11A, TNFRSF11B, TNFRSF12, TNFRSF12L, TNFRSF13, TNFRSF13B,

TNFRSF14, TNFRSF15, TNFRSF16, TNFRSF17, TNFRSF18, TNFRSF19, and TNFRSF20.

7. The fusion polypeptide of claim 6, wherein the polypeptide is capable of inhibiting signaling of a tumor necrosis family receptor super family signaling.
8. The fusion polypeptide of claim 7, wherein said first and second polypeptides of said fusion polypeptide are encoded by nucleic acids derived from a human.
9. The fusion polypeptide of claim 8, wherein said fusion polypeptide is non-immunogenic.
10. The fusion polypeptide of claim 5, wherein the second polypeptide is a soluble portion of a polypeptide selected from the group consisting of TNFSF1, TNFSF2, TNFSF3, TNFSF4, TNFSF5, TNFSF6, TNFSF7, TNFSF8, TNFSF9, TNFSF10, TNFSF11, TNFSF12, TNFSF13, TNFSF14, TNFSF15, TNFSF16, TNFSF17, TNFSF18, TNFSF19, and TNFSF20.
11. The fusion polypeptide of claim 10, wherein tumor necrosis family receptor super family signaling is enhanced.
12. The fusion polypeptide of claim 11, wherein said first and second polypeptides of said fusion polypeptide are encoded by nucleic acids derived from a human.
13. The fusion polypeptide of claim 12, wherein said fusion polypeptide is capable of forming a multimer of a trimer.

14. The fusion polypeptide of claim 13, wherein the second heterologous polypeptide comprises the amino acids depicted as amino acids 96 to 244 of SEQ ID NO:6.
15. A nucleic acid encoding the polypeptide of any one of claims 1, 2 or 3.
16. A host cell comprising a nucleic acid of claim 15, wherein said nucleic acid is operably linked to a heterologous control element.
17. A host cell transfected with a nucleic acid according to claim 15.
18. A method of producing a polypeptide encoded by the nucleic acid of claim 15, comprising the steps of growing a host cell transfected with said nucleic acid in conditions favorable for expression of the polypeptide, and isolating said polypeptide.
19. A recombinant vector comprising the nucleic acid of claim 15.
20. A method of constructing a recombinant expression vector comprising the steps of linking a first nucleic acid comprising nucleotides x to y of the nucleic acid depicted in SEQ ID NO:9, wherein x is 331 to 508, and y is 546 to 808, and a second nucleic acid encoding a heterologous polypeptide to the first nucleic acid into a compatible cloning site of an expression vector, followed by amplifying and isolating said recombinant expression vector.
21. The method of claim 20, wherein the second nucleic acid encodes a soluble portion of a polypeptide selected from the group consisting of TNFSF1, TNFRSF1A, TNFSF2, TNFRSF1B, TNFSF3, TNFRSF3, TNFSF4, TNFRSF4, TNFSF5, TNFRSF5, TNFSF6, TNFRSF6, TNFRSF6B, TNFSF7, TNFRSF7, TNFSF8, TNFRSF8, TNFSF9, TNFRSF9, TNFSF10, TNFRSF10A, TNFRSF10B, TNFRSF10C, TNFRSF10D, TNFSF11, TNFRSF11A, TNFRSF11B, TNFSF12, TNFRSF12, TNFRSF12L, TNFSF13, TNFRSF13, TNFSF13B, TNFSF14, TNFRSF14, TNFSF15,

TNFRSF15, TNFSF16, NFRSF16, TNFSF17, TNFRSF17, TNFSF18, TNFRSF18,
TNFSF19, TNFRSF19, TNFSF20, and TNFRSF20.

Figure 1

SR L2 (34)

CTAGAGCTCCTGGTGAAGATTGGATTTTGCCATTCAGATTTTCTATGTTGAGC
TGCAAATCAAGCAATGTGGTATTCAAA

SR U2 (33)

AGTCCTTAATAAGTTTGAATACCACATTGCTTGATTTGCAGCTCAACATAGAA
AATCTGAATGGCAAAATCCAATCTTCACCAGGAGCT

SR U1 (32)

CTAGGTCTGACATTCTTCTGCAGCTAAGTACCTTGTTTTCTCAGTCCAGGGA
CATGGGAATGCAATAGATGAAATCTCCA

SR L1 (35)

CTTATTAAGGACTTGGAGATTTTCATCTATTGCATTCCCATGTCCCTGGACTGA
GGAAAACAAGGTACTTAGCTGCAGAAGAATGTCAGAC

CTAGGTCTGACATTCTTCTGCAGCTAAGTACCTTGTTTTCTCAGTCCAGGGA
CATGGGAATGCAATAGATGAAATCTCCAAGTCCTTAATAAGTTTGAATACCA
CATTGCTTGATTTGCAGCTCAACATAGAAAATCTGAATGGCAAAATCCAATCT
TCACCAGGAGCTCTAG

Figure 2A

```

1/1                               31/11
atg gcc ctg tgg atc gac agg atg caa ctc ctg tct tgc att gca cta agt ctt gca ctt
M A L W I D R M Q L L S C I A L S L A L
61/21                               91/31
gtc aca aac agt gca cct act tca agt tct aca aag aaa aca cag cta act agg tct GAC
V T N S A P T S S S T K K T Q L T R S D
121/41                               151/51
ATT CTT CTG CAG CTA AGT ACC TTG TTT TCC TCA GTC CAG GGA CAT GGG AAT GCA ATA GAT
I L L Q L S T L F S S V Q G H G N A I D
181/61                               211/71
GAA ATC TCC AAG TCC TTA ATA AGT TTG AAT ACC ACA TTG CTT GAT TTG CAG CTC AAC ATA
E I S K S L I S L N T T L L D L Q L N I
241/81                               271/91
GAA AAT CTG AAT GGC AAA ATC CAA tct tca cca gga gct cta gaa ATG CAA AAA GGT GAT
E N L N G K I Q S S P G A L E M Q K G D
301/101                               331/111
CAG AAT CCT CAA ATT GCG GCA CAT GTC ATA AGT GAG GCC AGC AGT AAA ACA ACA TCT GTG
Q N P Q I A A H V I S E A S S K T T S V
361/121                               391/131
TTA CAG TGG GCT GAA AAA GGA TAC TAC ACC ATG AGC AAC AAC TTG GTA ACC CTG GAA AAT
L Q W A E K G Y Y T M S N N L V T L E N
421/141                               451/151
GGG AAA CAG CTG acc GTT AAA AGA CAA GGA CTC TAT TAT ATC TAT GCC CAA GTC ACC TTC
G K Q L T V K R Q G L Y Y I Y A Q V T F
481/161                               511/171
TGT TCC AAT CGG GAA GCT TCG AGT CAA GCT CCA TTT ATA GCC AGC CTC TGG CTA AAG TCC
C S N R E A S S Q A P F I A S L W L K S
541/181                               571/191
CCC GGT AGA TTC GAG AGA ATC TTA CTC AGA GCT GCA AAT ACC CAC AGT TCC GCC AAA CCT
P G R F E R I L L R A A N T H S S A K P
601/201                               631/211
TGC GGG CAA CAA TCC ATT CAC TTG GGA GGA GTA TTT GAA TTG CAA CCA GGT GCT TCG GTG
C G Q Q S I H L G G V F E L Q P G A S V
661/221                               691/231
TTT GTC AAT GTG ACT GAT CCA AGC CAA GTG AGC CAT GGC ACT GGC TTC ACG TCC TTT GGC
F V N V T D P S Q V S H G T G F T S F G
721/241
TTA CTC AAA CTC TGA GcG GcC gc
L L K L * A A

```

Figure 2B

```

1/1                               31/11
atg gct aca ggc tcc cgg acg tcc ctg ctc ctg gct ttt ggc ctg ctc tgc ctg ccc tgg
M A T G S R T S L L L A F G L L C L P W
61/21                               91/31
ctt caa gag ggc agt gca act agg tct GAC ATT CTT CTG CAG CTA AGT ACC TTG TTT TCC
L Q E G S A T R S D I L L Q L S T L F S
121/41                               151/51
TCA GTC CAG GGA CAT GGG AAT GCA ATA GAT GAA ATC TCC AAG TCC TTA ATA AGT TTG AAT
S V Q G H G N A I D E I S K S L I S L N
181/61                               211/71
ACC ACA TTG CTT GAT TTG CAG CTC AAC ATA GAA AAT CTG AAT GGC AAA ATC CAA tct tca
T T L L D L Q L N I E N L N G K I Q S S
241/81                               271/91
cca gga gct cta gaa ATG CAA AAA GGT GAT CAG AAT CCT CAA ATT GCG GCA CAT GTC ATA
P G A L E M Q K G D Q N P Q I A A H V I
301/101                               331/111
AGT GAG GCC AGC AGT AAA ACA ACA TCT GTG TTA CAG TGG GCT GAA AAA GGA TAC TAC ACC
S E A S S K T T S V L Q W A E K G Y Y T
361/121                               391/131
ATG AGC AAC AAC TTG GTA ACC CTG GAA AAT GGG AAA CAG CTG aCC GTT AAA AGA CAA GGA
M S N N L V T L E N G K Q L T V K R Q G
421/141                               451/151
CTC TAT TAT ATC TAT GCC CAA GTC ACC TTC TGT TCC AAT CCG GAA GCT TCG AGT CAA GCT
L Y Y I Y A Q V T F C S N R E A S S Q A
481/161                               511/171
CCA TTT ATA GCC AGC CTC TGG CTA AAG TCC CCC GGT AGA TTC GAG AGA ATC TTA CTC AGA
P F I A S L W L K S P G R F E R I L L R
541/181                               571/191
GCT GCA AAT ACC CAC AGT TCC GCC AAA CCT TGC GGG CAA CAA TCC ATT CAC TTG GGA GGA
A A N T H S S A K P C G Q Q S I H L G G
601/201                               631/211
GTA TTT GAA TTG CAA CCA GGT GCT TCG GTG TTT GTC AAT GTG ACT GAT CCA AGC CAA GTG
V F E L Q P G A S V F V N V T D P S Q V
661/221                               691/231
AGC CAT GGC ACT GGC TTC ACG TCC TTT GGC TTA CTC AAA CTC TGA GcG GcC gc
S H G T G F T S F G L L K L * A A

```


Figure 3

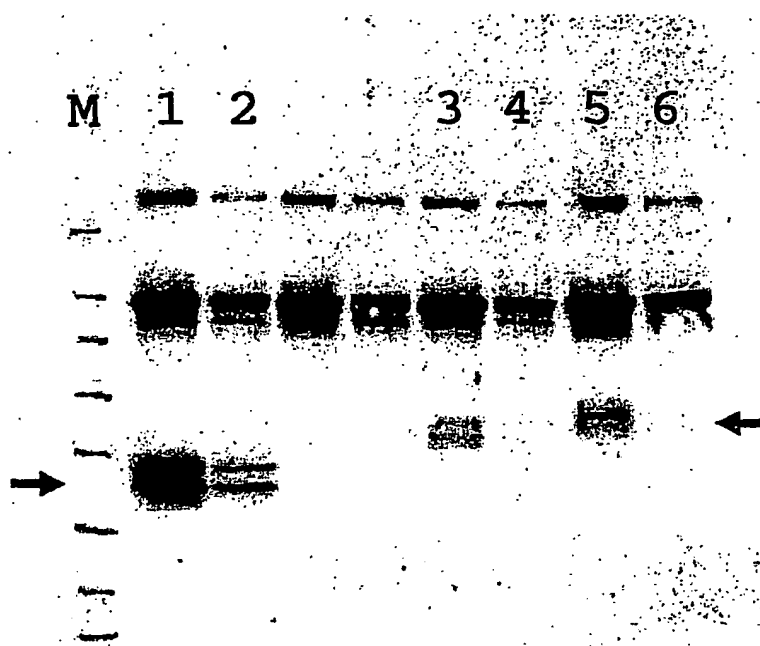


Figure 4

```

      10      20      30      40      50
-----|-----|-----|-----|
Query : ATGGAGCAGTGGGATCACTTTCACAATCAACAGGAGGACACTGATAGCTG
Frame1 : M E Q W D H F H N Q Q E D T D S C

      60      70      80      90      100
-----|-----|-----|-----|
Query : CTCCGAATCTGTGAAATTTGATGCTCGCTCAATGACAGCTTTGCTTCCTC
Frame1 : S E S V K F D A R S M T A L L P P

     110     120     130     140     150
-----|-----|-----|-----|
Query : CGAATCCTAAAAACAGCCCTTCCCTTCAAGAGAACTGAAGTCCTTCAAA
Frame1 : N P K N S P S L Q E K L K S F K

     160     170     180     190     200
-----|-----|-----|-----|
Query : GCTGCACTGATTGCCCTTTACCTCCTCGTGTTCAGTTCTCATCCCTCT
Frame1 : A A L I A L Y L L V F A V L I P L

     210     220     230     240     250
-----|-----|-----|-----|
Query : CATTGAATAGTGGCAGCTCAACTCCTGAAGTGGGAAACGAAGAATTGCT
Frame1 : I G I V A A Q L L K W E T K N C S

     260     270     280     290     300
-----|-----|-----|-----|
Query : CAGTTAGTTCAACTAATGCAAATGATATAACTCAAAGTCTCACGGGAAAA
Frame1 : V S S T N A N D I T Q S L T G K

     310     320     330     340     350
-----|-----|-----|-----|
Query : GGAAATGACAGCGAAGAGGAAATGAGATTTCAAGAAGTCTTTATGGAACA
Frame1 : G N D S E E E M R F Q E V F M E H

     360     370     380     390     400
-----|-----|-----|-----|
Query : CATGAGCAACATGGAGAAGAGAATCCAGCATATTTAGACATGGAAGCCA
Frame1 : M S N M E K R I Q H I L D M E A N

     410     420     430     440     450
-----|-----|-----|-----|
Query : ACCTCATGGACACAGAGCATTTCCAAAATTTAGCATGACAACCTGATCAA
Frame1 : L M D T E H F Q N F S M T T D Q

     460     470     480     490     500
-----|-----|-----|-----|
Query : AGATTTAATGACATTCTTCTGCAGCTAAGTACCTTGTTCCTCAGTCCA

```

Frame1 : R F N D I L L Q L S T L F S S V Q

510 520 530 540 550
 -----|-----|-----|-----|-----|
 Query : GGGACATGGGAATGCAATAGATGAAATCTCCAAGTCCTTAATAAGTTTGA
 Frame1 : G H G N A I D E I S K S L I S L N

560 570 580 590 600
 -----|-----|-----|-----|-----|
 Query : ATACCACATTGCTTGATTGTCAGCTCAACATAGAAAATCTGAATGGCAAA
 Frame1 : T T L L D L Q L N I E N L N G K

610 620 630 640 650
 -----|-----|-----|-----|-----|
 Query : ATCCAAGAGAATACCTTCAAACAACAAGAGGAAATCAGTAAATTAGAGGA
 Frame1 : I Q E N T F K Q Q E E I S K L E E

660 670 680 690 700
 -----|-----|-----|-----|-----|
 Query : GCGTGTTTACAATGTATCAGCAGAAATTATGGCTATGAAAGAAGAAACAAG
 Frame1 : R V Y N V S A E I M A M K E E Q V

710 720 730 740 750
 -----|-----|-----|-----|-----|
 Query : TGCATTGGAACAGGAAATAAAAGGAGAAGTGAAAGTACTGAATAACATC
 Frame1 : H L E Q E I K G E V K V L N N I

760 770 780 790 800
 -----|-----|-----|-----|-----|
 Query : ACTAATGATCTCAGACTGAAAGATTGGGAACATTCTCAGACCTTGAGAAA
 Frame1 : T N D L R L K D W E H S Q T L R N

810 820 830 840 850
 -----|-----|-----|-----|-----|
 Query : TATCACTTTAATTCAAGGTCCTCCTGGACCCCGGGTGAAAAAGGAGATC
 Frame1 : I T L I Q G P P G P P G E K G D R

860 870 880 890 900
 -----|-----|-----|-----|-----|
 Query : GAGGTCCCACTGGAGAAAGTGGTCCACGAGGATTTCCAGGTCCAATAGGT
 Frame1 : G P T G E S G P R G F P G P I G

910 920 930 940 950
 -----|-----|-----|-----|-----|
 Query : CCTCCGGGTCTTAAAGGTGATCGGGGAGCAATTGGCTTTCTGGAAGTCG
 Frame1 : P P G L K G D R G A I G F P G S R

960 970 980 990 1000
 -----|-----|-----|-----|-----|
 Query : AGGACTCCCAGGATATGCCGGAAGGCCAGGAAATTCTGGACCAAAGGCC
 Frame1 : G L P G Y A G R P G N S G P K G Q

```

               1010       1020       1030       1040       1050
               -----|-----|-----|-----|
Query  : AGAAAGGGGAAAAGGGGAGTGGAAACACATTAAGACCAGTACAACCTCACT
Frame1 :  K G E K G S G N T L R P V Q L T

               1060       1070
               -----|-----|
Query  : GATCATATTAGGGCAGGGCCCTCTTAA
Frame1 : D H I R A G P S *
```

Seq List 3398.ST25.txt
SEQUENCE LISTING

<110> Reddy, Pranhitha

<120> Trimeric Recombinant Polypeptides

<130> 3398-WO

<160> 10

<170> PatentIn version 3.1

<210> 1

<211> 80

<212> DNA

<213> Homo sapiens

<400> 1

ctagagctcc tggatgaagat tggattttgc cattcagatt ttctatgttg agctgcaaat 60

caagcaatgt ggtattcaaa 80

<210> 2

<211> 89

<212> DNA

<213> Homo sapiens

<400> 2

agtccttaat aagtttgaat accacattgc ttgatttgca gctcaacata gaaaatctga 60

atggcaaaat ccaatcttca ccaggagct 89

<210> 3

<211> 81

<212> DNA

<213> Homo sapiens

<400> 3

ctaggctctga cattcttctg cagctaagta ccttgttttc ctcagtccag ggacatggga 60

atgcaataga tgaaatctcc a 81

<210> 4

<211> 90

<212> DNA

<213> Homo sapiens

<400> 4

cttattaagg acttggagat ttcatctatt gcattcccat gtccctggac tgaggaaaac 60

aaggtactta gctgcagaag aatgtcagac 90

<210> 5

<211> 1077

<212> DNA

<213> Homo sapiens

<220>

<221> CDS

Seq List 3398.ST25.txt

<222> (1)..(1077)

<223>

<400> 5

atg	gag	cag	tgg	gat	cac	ttt	cac	aat	caa	cag	gag	gac	act	gat	agc	48
Met	Glu	Gln	Trp	Asp	His	Phe	His	Asn	Gln	Gln	Glu	Asp	Thr	Asp	Ser	
1				5					10					15		
tgc	tcc	gaa	tct	gtg	aaa	ttt	gat	gct	cgc	tca	atg	aca	gct	ttg	ctt	96
Cys	Ser	Glu	Ser	Val	Lys	Phe	Asp	Ala	Arg	Ser	Met	Thr	Ala	Leu	Leu	
			20					25					30			
cct	ccg	aat	cct	aaa	aac	agc	cct	tcc	ctt	caa	gag	aaa	ctg	aag	tcc	144
Pro	Pro	Asn	Pro	Lys	Asn	Ser	Pro	Ser	Leu	Gln	Glu	Lys	Leu	Lys	Ser	
		35					40					45				
ttc	aaa	gct	gca	ctg	att	gcc	ctt	tac	ctc	ctc	gtg	ttt	gca	gtt	ctc	192
Phe	Lys	Ala	Ala	Leu	Ile	Ala	Leu	Tyr	Leu	Leu	Val	Phe	Ala	Val	Leu	
	50					55					60					
atc	cct	ctc	att	gga	ata	gtg	gca	gct	caa	ctc	ctg	aag	tgg	gaa	acg	240
Ile	Pro	Leu	Ile	Gly	Ile	Val	Ala	Ala	Gln	Leu	Leu	Lys	Trp	Glu	Thr	
65				70					75					80		
aag	aat	tgc	tca	gtt	agt	tca	act	aat	gca	aat	gat	ata	act	caa	agt	288
Lys	Asn	Cys	Ser	Val	Ser	Ser	Thr	Asn	Ala	Asn	Asp	Ile	Thr	Gln	Ser	
				85					90					95		
ctc	acg	gga	aaa	gga	aat	gac	agc	gaa	gag	gaa	atg	aga	ttt	caa	gaa	336
Leu	Thr	Gly	Lys	Gly	Asn	Asp	Ser	Glu	Glu	Glu	Met	Arg	Phe	Gln	Glu	
			100					105					110			
gtc	ttt	atg	gaa	cac	atg	agc	aac	atg	gag	aag	aga	atc	cag	cat	att	384
Val	Phe	Met	Glu	His	Met	Ser	Asn	Met	Glu	Lys	Arg	Ile	Gln	His	Ile	
		115					120					125				
tta	gac	atg	gaa	gcc	aac	ctc	atg	gac	aca	gag	cat	ttc	caa	aat	ttc	432
Leu	Asp	Met	Glu	Ala	Asn	Leu	Met	Asp	Thr	Glu	His	Phe	Gln	Asn	Phe	
	130					135						140				
agc	atg	aca	act	gat	caa	aga	ttt	aat	gac	att	ctt	ctg	cag	cta	agt	480
Ser	Met	Thr	Thr	Asp	Gln	Arg	Phe	Asn	Asp	Ile	Leu	Leu	Gln	Leu	Ser	
145					150					155				160		
acc	ttg	ttt	tcc	tca	gtc	cag	gga	cat	ggg	aat	gca	ata	gat	gaa	atc	528
Thr	Leu	Phe	Ser	Ser	Val	Gln	Gly	His	Gly	Asn	Ala	Ile	Asp	Glu	Ile	
				165					170					175		
tcc	aag	tcc	tta	ata	agt	ttg	aat	acc	aca	ttg	ctt	gat	ttg	cag	ctc	576
Ser	Lys	Ser	Leu	Ile	Ser	Leu	Asn	Thr	Thr	Leu	Leu	Asp	Leu	Gln	Leu	
			180					185					190			
aac	ata	gaa	aat	ctg	aat	ggc	aaa	atc	caa	gag	aat	acc	ttc	aaa	caa	624
Asn	Ile	Glu	Asn	Leu	Asn	Gly	Lys	Ile	Gln	Glu	Asn	Thr	Phe	Lys	Gln	
		195					200					205				
caa	gag	gaa	atc	agt	aaa	tta	gag	gag	cgt	gtt	tac	aat	gta	tca	gca	672
Gln	Glu	Glu	Ile	Ser	Lys	Leu	Glu	Glu	Arg	Val	Tyr	Asn	Val	Ser	Ala	
	210					215					220					
gaa	att	atg	gct	atg	aaa	gaa	gaa	caa	gtg	cat	ttg	gaa	cag	gaa	ata	720
Glu	Ile	Met	Ala	Met	Lys	Glu	Glu	Gln	Val	His	Leu	Glu	Gln	Glu	Ile	
225					230					235				240		

Seq List 3398.ST25.txt

aaa gga gaa gtg aaa gta ctg aat aac atc act aat gat ctc aga ctg 768
 Lys Gly Glu Val Lys Val Leu Asn Asn Ile Thr Asn Asp Leu Arg Leu
 245 250 255
 aaa gat tgg gaa cat tct cag acc ttg aga aat atc act tta att caa 816
 Lys Asp Trp Glu His Ser Gln Thr Leu Arg Asn Ile Thr Leu Ile Gln
 260 265 270
 ggt cct cct gga ccc ccg ggt gaa aaa gga gat cga ggt ccc act gga 864
 Gly Pro Pro Gly Pro Pro Gly Glu Lys Gly Asp Arg Gly Pro Thr Gly
 275 280 285
 gaa agt ggt cca cga gga ttt cca ggt cca ata ggt cct ccg ggt ctt 912
 Glu Ser Gly Pro Arg Gly Phe Pro Gly Pro Ile Gly Pro Pro Gly Leu
 290 295 300
 aaa ggt gat cgg gga gca att ggc ttt cct gga agt cga gga ctc cca 960
 Lys Gly Asp Arg Gly Ala Ile Gly Phe Pro Gly Ser Arg Gly Leu Pro
 305 310 315 320
 gga tat gcc gga agg cca gga aat tct gga cca aaa ggc cag aaa ggg 1008
 Gly Tyr Ala Gly Arg Pro Gly Asn Ser Gly Pro Lys Gly Gln Lys Gly
 325 330 335
 gaa aag ggg agt gga aac aca tta aga cca gta caa ctc act gat cat 1056
 Glu Lys Gly Ser Gly Asn Thr Leu Arg Pro Val Gln Leu Thr Asp His
 340 345 350
 att agg gca ggg ccc tct taa 1077
 Ile Arg Ala Gly Pro Ser
 355

<210> 6
 <211> 358
 <212> PRT
 <213> Homo sapiens

<400> 6

Met Glu Gln Trp Asp His Phe His Asn Gln Gln Glu Asp Thr Asp Ser
1 5 10 15

Cys Ser Glu Ser Val Lys Phe Asp Ala Arg Ser Met Thr Ala Leu Leu
20 25 30

Pro Pro Asn Pro Lys Asn Ser Pro Ser Leu Gln Glu Lys Leu Lys Ser
35 40 45

Phe Lys Ala Ala Leu Ile Ala Leu Tyr Leu Leu Val Phe Ala Val Leu
50 55 60

Ile Pro Leu Ile Gly Ile Val Ala Ala Gln Leu Leu Lys Trp Glu Thr
65 70 75 80

Lys Asn Cys Ser Val Ser Ser Thr Asn Ala Asn Asp Ile Thr Gln Ser
85 90 95

Seq List 3398.ST25.txt

Leu Thr Gly Lys Gly Asn Asp Ser Glu Glu Glu Met Arg Phe Gln Glu
100 105 110

Val Phe Met Glu His Met Ser Asn Met Glu Lys Arg Ile Gln His Ile
115 120 125

Leu Asp Met Glu Ala Asn Leu Met Asp Thr Glu His Phe Gln Asn Phe
130 135 140

Ser Met Thr Thr Asp Gln Arg Phe Asn Asp Ile Leu Leu Gln Leu Ser
145 150 155 160

Thr Leu Phe Ser Ser Val Gln Gly His Gly Asn Ala Ile Asp Glu Ile
165 170 175

Ser Lys Ser Leu Ile Ser Leu Asn Thr Thr Leu Leu Asp Leu Gln Leu
180 185 190

Asn Ile Glu Asn Leu Asn Gly Lys Ile Gln Glu Asn Thr Phe Lys Gln
195 200 205

Gln Glu Glu Ile Ser Lys Leu Glu Glu Arg Val Tyr Asn Val Ser Ala
210 215 220

Glu Ile Met Ala Met Lys Glu Glu Gln Val His Leu Glu Gln Glu Ile
225 230 235 240

Lys Gly Glu Val Lys Val Leu Asn Asn Ile Thr Asn Asp Leu Arg Leu
245 250 255

Lys Asp Trp Glu His Ser Gln Thr Leu Arg Asn Ile Thr Leu Ile Gln
260 265 270

Gly Pro Pro Gly Pro Pro Gly Glu Lys Gly Asp Arg Gly Pro Thr Gly
275 280 285

Glu Ser Gly Pro Arg Gly Phe Pro Gly Pro Ile Gly Pro Pro Gly Leu
290 295 300

Lys Gly Asp Arg Gly Ala Ile Gly Phe Pro Gly Ser Arg Gly Leu Pro
305 310 315 320

Gly Tyr Ala Gly Arg Pro Gly Asn Ser Gly Pro Lys Gly Gln Lys Gly
325 330 335

Glu Lys Gly Ser Gly Asn Thr Leu Arg Pro Val Gln Leu Thr Asp His
Page 4

340

Seq List 3398.ST25.txt
345 350Ile Arg Ala Gly Pro Ser
355

<210> 7
 <211> 743
 <212> DNA
 <213> Homo sapiens

<220>
 <221> CDS
 <222> (1)..(735)
 <223>

<400> 7
 atg gcc ctg tgg atc gac agg atg caa ctc ctg tct tgc att gca cta 48
 Met Ala Leu Trp Ile Asp Arg Met Gln Leu Ser Cys Ile Ala Leu
 1 5 10 15
 agt ctt gca ctt gtc aca aac agt gca cct act tca agt tct aca aag 96
 Ser Leu Ala Leu Val Thr Asn Ser Ala Pro Thr Ser Ser Thr Lys
 20 25 30
 aaa aca cag cta act agg tct gac att ctt ctg cag cta agt acc ttg 144
 Lys Thr Gln Leu Thr Arg Ser Asp Ile Leu Leu Gln Leu Ser Thr Leu
 35 40 45
 ttt tcc tca gtc cag gga cat ggg aat gca ata gat gaa atc tcc aag 192
 Phe Ser Ser Val Gln Gly His Gly Asn Ala Ile Asp Glu Ile Ser Lys
 50 55 60
 tcc tta ata agt ttg aat acc aca ttg ctt gat ttg cag ctc aac ata 240
 Ser Leu Ile Ser Leu Asn Thr Thr Leu Leu Asp Leu Gln Leu Asn Ile
 65 70 75 80
 gaa aat ctg aat ggc aaa atc caa tct tca cca gga gct cta gaa atg 288
 Glu Asn Leu Asn Gly Lys Ile Gln Ser Ser Pro Gly Ala Leu Glu Met
 85 90 95
 caa aaa ggt gat cag aat cct caa att gcg gca cat gtc ata agt gag 336
 Gln Lys Gly Asp Gln Asn Pro Gln Ile Ala Ala His Val Ile Ser Glu
 100 105 110
 gcc agc agt aaa aca aca tct gtg tta cag tgg gct gaa aaa gga tac 384
 Ala Ser Ser Lys Thr Thr Ser Val Leu Gln Trp Ala Glu Lys Gly Tyr
 115 120 125
 tac acc atg agc aac aac ttg gta acc ctg gaa aat ggg aaa cag ctg 432
 Tyr Thr Met Ser Asn Asn Leu Val Thr Leu Glu Asn Gly Lys Gln Leu
 130 135 140
 acc gtt aaa aga caa gga ctc tat tat atc tat gcc caa gtc acc ttc 480
 Thr Val Lys Arg Gln Gly Leu Tyr Tyr Ile Tyr Ala Gln Val Thr Phe
 145 150 155 160
 tgt tcc aat cgg gaa gct tcg agt caa gct cca ttt ata gcc agc ctc 528
 Cys Ser Asn Arg Glu Ala Ser Ser Gln Ala Pro Phe Ile Ala Ser Leu
 165 170 175
 tgg cta aag tcc ccc ggt aga ttc gag aga atc tta ctc aga gct gca 576

Seq List 3398.ST25.txt

Trp	Leu	Lys	Ser	Pro	Gly	Arg	Phe	Glu	Arg	Ile	Leu	Leu	Arg	Ala	Ala		
			180					185					190				
aat	acc	cac	agt	tcc	gcc	aaa	cct	tgc	ggg	caa	caa	tcc	att	cac	ttg		624
Asn	Thr	His	Ser	Ser	Ala	Lys	Pro	Cys	Gly	Gln	Gln	Ser	Ile	His	Leu		
		195					200					205					
gga	gga	gta	ttt	gaa	ttg	caa	cca	ggt	gct	tcg	gtg	ttt	gtc	aat	gtg		672
Gly	Gly	Val	Phe	Glu	Leu	Gln	Pro	Gly	Ala	Ser	Val	Phe	Val	Asn	Val		
	210					215					220						
act	gat	cca	agc	caa	gtg	agc	cat	ggc	act	ggc	ttc	acg	tcc	ttt	ggc		720
Thr	Asp	Pro	Ser	Gln	Val	Ser	His	Gly	Thr	Gly	Phe	Thr	Ser	Phe	Gly		
225					230					235					240		
tta	ctc	aaa	ctc	tga	gcggccgc												743
Leu	Leu	Lys	Leu														

<210> 8
 <211> 244
 <212> PRT
 <213> Homo sapiens

<400> 8

Met	Ala	Leu	Trp	Ile	Asp	Arg	Met	Gln	Leu	Leu	Ser	Cys	Ile	Ala	Leu		
1				5				10						15			
Ser	Leu	Ala	Leu	Val	Thr	Asn	Ser	Ala	Pro	Thr	Ser	Ser	Ser	Thr	Lys		
			20					25					30				
Lys	Thr	Gln	Leu	Thr	Arg	Ser	Asp	Ile	Leu	Leu	Gln	Leu	Ser	Thr	Leu		
		35					40					45					
Phe	Ser	Ser	Val	Gln	Gly	His	Gly	Asn	Ala	Ile	Asp	Glu	Ile	Ser	Lys		
	50					55					60						
Ser	Leu	Ile	Ser	Leu	Asn	Thr	Thr	Leu	Leu	Asp	Leu	Gln	Leu	Asn	Ile		
65					70					75					80		
Glu	Asn	Leu	Asn	Gly	Lys	Ile	Gln	Ser	Ser	Pro	Gly	Ala	Leu	Glu	Met		
			85						90					95			
Gln	Lys	Gly	Asp	Gln	Asn	Pro	Gln	Ile	Ala	Ala	His	Val	Ile	Ser	Glu		
			100					105					110				
Ala	Ser	Ser	Lys	Thr	Thr	Ser	Val	Leu	Gln	Trp	Ala	Glu	Lys	Gly	Tyr		
		115					120					125					
Tyr	Thr	Met	Ser	Asn	Asn	Leu	Val	Thr	Leu	Glu	Asn	Gly	Lys	Gln	Leu		
130						135					140						

Seq List 3398.ST25.txt

Thr Val Lys Arg Gln Gly Leu Tyr Tyr Ile Tyr Ala Gln Val Thr Phe
 145 150 155 160

Cys Ser Asn Arg Glu Ala Ser Ser Gln Ala Pro Phe Ile Ala Ser Leu
 165 170 175

Trp Leu Lys Ser Pro Gly Arg Phe Glu Arg Ile Leu Leu Arg Ala Ala
 180 185 190

Asn Thr His Ser Ser Ala Lys Pro Cys Gly Gln Gln Ser Ile His Leu
 195 200 205

Gly Gly Val Phe Glu Leu Gln Pro Gly Ala Ser Val Phe Val Asn Val
 210 215 220

Thr Asp Pro Ser Gln Val Ser His Gly Thr Gly Phe Thr Ser Phe Gly
 225 230 235 240

Leu Leu Lys Leu

<210> 9
 <211> 713
 <212> DNA
 <213> Homo sapiens

<220>
 <221> CDS
 <222> (1)..(705)
 <223>

<400> 9
 atg gct aca ggc tcc cgg acg tcc ctg ctc ctg gct ttt ggc ctg ctc 48
 Met Ala Thr Gly Ser Arg Thr Ser Leu Leu Leu Ala Phe Gly Leu Leu
 1 5 10 15

tgc ctg ccc tgg ctt caa gag ggc agt gca act agg tct gac att ctt 96
 Cys Leu Pro Trp Leu Gln Glu Gly Ser Ala Thr Arg Ser Asp Ile Leu
 20 25 30

ctg cag cta agt acc ttg ttt tcc tca gtc cag gga cat ggg aat gca 144
 Leu Gln Leu Ser Thr Leu Phe Ser Val Gln Gly His Gly Asn Ala
 35 40 45

ata gat gaa atc tcc aag tcc tta ata agt ttg aat acc aca ttg ctt 192
 Ile Asp Glu Ile Ser Lys Ser Leu Ile Ser Leu Asn Thr Thr Leu Leu
 50 55 60

gat ttg cag ctc aac ata gaa aat ctg aat ggc aaa atc caa tct tca 240
 Asp Leu Gln Leu Asn Ile Glu Asn Leu Asn Gly Lys Ile Gln Ser Ser
 65 70 75 80

cca gga gct cta gaa atg caa aaa ggt gat cag aat cct caa att gcg 288
 Pro Gly Ala Leu Glu Met Gln Lys Gly Asp Gln Asn Pro Gln Ile Ala
 85 90 95

Seq List 3398.ST25.txt

gca cat gtc ata agt gag gcc agc agt aaa aca aca tct gtg tta cag	336
Ala His Val Ile Ser Glu Ala Ser Ser Lys Thr Thr Ser Val Leu Gln	
100 105 110	
tggt gct gaa aaa gga tac tac acc atg agc aac aac ttg gta acc ctg	384
Trp Ala Glu Lys Gly Tyr Tyr Thr Met Ser Asn Asn Leu Val Thr Leu	
115 120 125	
gaa aat ggg aaa cag ctg acc gtt aaa aga caa gga ctc tat tat atc	432
Glu Asn Gly Lys Gln Leu Thr Val Lys Arg Gln Gly Leu Tyr Tyr Ile	
130 135 140	
tat gcc caa gtc acc ttc tgt tcc aat cgg gaa gct tcg agt caa gct	480
Tyr Ala Gln Val Thr Phe Cys Ser Asn Arg Glu Ala Ser Ser Gln Ala	
145 150 155 160	
cca ttt ata gcc agc ctc tgg cta aag tcc ccc ggt aga ttc gag aga	528
Pro Phe Ile Ala Ser Leu Trp Leu Lys Ser Pro Gly Arg Phe Glu Arg	
165 170 175	
atc tta ctc aga gct gca aat acc cac agt tcc gcc aaa cct tgc ggg	576
Ile Leu Leu Arg Ala Ala Asn Thr His Ser Ser Ala Lys Pro Cys Gly	
180 185 190	
caa caa tcc att cac ttg gga gga gta ttt gaa ttg caa caa ggt gct	624
Gln Gln Ser Ile His Leu Gly Gly Val Phe Glu Leu Gln Gln Gly Ala	
195 200 205	
tcg gtg ttt gtc aat gtg act gat cca agc caa gtg agc cat ggc act	672
Ser Val Phe Val Asn Val Thr Asp Pro Ser Gln Val Ser His Gly Thr	
210 215 220	
ggc ttc acg tcc ttt ggc tta ctc aaa ctc tga gcggccgc	713
Gly Phe Thr Ser Phe Gly Leu Leu Lys Leu	
225 230	

<210> 10
 <211> 234
 <212> PRT
 <213> Homo sapiens

<400> 10

Met Ala Thr Gly Ser Arg Thr Ser Leu Leu Leu Ala Phe Gly Leu Leu
 1 5 10 15

Cys Leu Pro Trp Leu Gln Glu Gly Ser Ala Thr Arg Ser Asp Ile Leu
 20 25 30

Leu Gln Leu Ser Thr Leu Phe Ser Ser Val Gln Gly His Gly Asn Ala
 35 40 45

Ile Asp Glu Ile Ser Lys Ser Leu Ile Ser Leu Asn Thr Thr Leu Leu
 50 55 60

Asp Leu Gln Leu Asn Ile Glu Asn Leu Asn Gly Lys Ile Gln Ser Ser
 65 70 75 80

Seq List 3398.ST25.txt

Pro Gly Ala Leu ⁸⁵Glu Met Gln Lys Gly ⁹⁰Asp Gln Asn Pro Gln ⁹⁵Ile Ala
 Ala His Val ¹⁰⁰Ile Ser Glu Ala Ser ¹⁰⁵Ser Lys Thr Thr Ser ¹¹⁰Val Leu Gln
 Trp Ala ¹¹⁵Glu Lys Gly Tyr Tyr ¹²⁰Thr Met Ser Asn Asn ¹²⁵Leu Val Thr Leu
 Glu ¹³⁰Asn Gly Lys Gln Leu ¹³⁵Thr Val Lys Arg Gln ¹⁴⁰Gly Leu Tyr Tyr Ile
 Tyr ¹⁴⁵Ala Gln Val Thr ¹⁵⁰Phe Cys Ser Asn Arg ¹⁵⁵Glu Ala Ser Ser Gln ¹⁶⁰Ala
 Pro Phe Ile Ala ¹⁶⁵Ser Leu Trp Leu Lys ¹⁷⁰Ser Pro Gly Arg Phe ¹⁷⁵Glu Arg
 Ile Leu Leu ¹⁸⁰Arg Ala Ala Asn Thr ¹⁸⁵His Ser Ser Ala Lys ¹⁹⁰Pro Cys Gly
 Gln Gln ¹⁹⁵Ser Ile His Leu Gly ²⁰⁰Gly Val Phe Glu Leu ²⁰⁵Gln Gln Gly Ala
 Ser ²¹⁰Val Phe Val Asn Val ²¹⁵Thr Asp Pro Ser Gln ²²⁰Val Ser His Gly Thr
 Gly ²²⁵Phe Thr Ser Phe ²³⁰Gly Leu Leu Lys Leu

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
24 July 2003 (24.07.2003)

PCT

(10) International Publication Number
WO 2003/060072 A3

(51) International Patent Classification⁷: **C07K 14/52**,
14/525, 14/705, 14/715, 14/725, C12N 5/10, 15/10, 15/12,
15/19, 15/28

(21) International Application Number:
PCT/US2002/040913

(22) International Filing Date:
20 December 2002 (20.12.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/343,315 21 December 2001 (21.12.2001) US

(71) Applicant (*for all designated States except US*): **IM-
MUNEX CORPORATION** [US/US]; Law Department,
51 University Street, Seattle, WA 98101 (US).

(72) Inventor; and

(75) Inventor/Applicant (*for US only*): **REDDY, Pranhitha**
[US/US]; 6811 18th Avenue NE, Seattle, WA 98115 (US).

(74) Agents: **ODRE, Steven, M.** et al.; Immunex Corporation,
Law Department, 51 University Street, Seattle, WA 98101
(US).

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU,
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU,
CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,
GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC,
LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW,
MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG,
SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN,
YU, ZA, ZM, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM,
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW),
Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),
European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE,
ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SI, SK,
TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ,
GW, ML, MR, NE, SN, TD, TG).

Published:

- with international search report
- before the expiration of the time limit for amending the
claims and to be republished in the event of receipt of
amendments

(88) Date of publication of the international search report:
15 January 2004

*For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.*

(54) Title: RECOMBINANT POLYPEPTIDES

(57) Abstract: The present invention relates, in part, to a polypeptide domain having reduced immunogenicity that is capable of inducing trimers in heterologous polypeptides, fusion polypeptides comprising the trimer inducing domain, nucleic acids encoding the polypeptides of the invention and methods of making and using the same.



WO 2003/060072 A3

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US02/40913

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C07K 14/52, 14/525, 14/705, 14/715, 14/725; C12N 5/10, 15/10, 15/12, 15/19, 15/28
 US CL : 530/350, 351; 536/23.1, 23.4, 23.5; 435/69.4, 320.1, 325, 252.3, 254.11

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/350, 351; 536/23.1, 23.4, 23.5; 435/69.4, 320.1, 325, 252.3, 254.11

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
 Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	FANSLOW ET AL. Structural characteristics of CD40 ligand that determine biological function. Seminars in Immunology. 1994, Vol. 6, pages 267-278, especially Figures 1 and 3.	1-3, 15-20 ----- 4-13, 21

Y		
X	MORRIS ET AL. Incorporation of an isoleucine zipper motif enhances the biological activity of soluble CD40L (CD154). J. Biol. Chem. 01 January 1999, Vol. 274, No. 1, pages 418-423, especially page 412.	1-3, 15-20 ----- 4-13, 21

Y		
Y	FRANK ET AL. A distinct seven-residue trigger sequence is indispensable for proper coiled-coil formation of the human macrophage scavenger receptor oligomerization domain. J. Biol. Chem. 21 April 2000, Vol. 275, No. 16, pages 11672-11677, see entire document.	4-13, 21
X	US 5,716,805 A (SRINIVASAN ET AL.) 10 February 1998 (10.02.1998), entire document.	1-13, 15-21
X	US 6,087,329 A (ARMITAGE ET AL.) 11 July 2000 (11.07.2000), entire document, especially TABLE 12.	1-3, 15-20 ----- 4-13, 21

Y		

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

Special categories of cited documents:	
* "A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"B" earlier application or patent published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

24 October 2003 (24.10.2003)

Date of mailing of the international search report

17 NOV 2003

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US
 Commissioner for Patents
 P.O. Box 1450
 Alexandria, Virginia 22313-1450
 Facsimile No. (703)305-3230

Authorized officer

Helicia L. Roberts
 Claire M. Kaufman

Telephone No. (703)308-0196

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US02/40913

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☒ Claim Nos.: 1-2,4-21 in part
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
Please See Continuation Sheet
3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
 2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
 3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
 4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
- Remark on Protest ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

PCT/US02/40913

Continuation of Box I Reason 2:

As SEQ ID NO:10 has only 234 amino acids and SEQ ID NO:9 has only 713 nucleotides, any values in the claims exceeding those lengths were unsearchable. Note that values equal to or less than those length were, however, able to be searched.

Continuation of B. FIELDS SEARCHED Item 3:

WEST, GenBank, EMBL, Medline, CaPlus

search terms: macrophage scavenger receptor, TNFRSF?, SR-A, CD40 or CD40L